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EDITORIAL

New perspectives on biological HDL-targeted therapies

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1. Introduction

High-density lipoproteins (HDL) consist of various subclasses, which share the abundance of apolipoprotein (apo) A-I, phospholipids, and cholesterol but are distinct by the variable presence of one or more representatives of at least 85 proteins and hundreds of lipid species [1,2]. HDL are circulating multimolecular platforms that exert divergent functions: reverse cholesterol transport, anti-inflammatory effects, antioxidative properties, immunomodulatory effects, improvement of endothelial function, antithrombotic effects, and potentiation of insulin secretion and improvement of insulin sensitivity [2].

Plasma HDL-cholesterol levels and plasma levels of its major apo, apo A-I, are inversely correlated with the incidence of ischemic cardiac diseases. A meta-analysis of four prospective studies indicated that a 1 mg/dL increase in HDL-cholesterol was associated with a 2% risk reduction of coronary heart disease in men and a 3% risk reduction in women [3]. In a more recent analysis of The Emerging Risk Factors Collaboration, the adjusted hazard ratio for coronary heart disease associated with a one standard deviation increase of HDL-cholesterol (15 mg/dL) was 0.78 (95% confidence interval, 0.74–0.82) [4]. In contrast, Mendelian randomization studies have demonstrated that genetic mechanisms that raise plasma HDL-cholesterol do not appear to lower the risk of myocardial infarction [5]. The possibility that the epidemiological relationship between HDL-cholesterol and coronary artery disease in classical epidemiological studies reflects residual confounding and/or unmeasured confounding cannot be excluded. Low HDL-cholesterol could be an integrated biomarker of adverse metabolic processes including abnormal metabolism of triglyceride-rich lipoproteins, insulin resistance, and ongoing tissue inflammation [2].

Based on the biological potential of HDL and on epidemiological evidence, the development of HDL-targeted therapies has been an important objective for several decades. According to the original HDL hypothesis, raising HDL-cholesterol was expected to lead to a decrease in coronary heart disease risk. However, HDL-cholesterol is a very poor proxy to analyze biological action and clinical effects of HDL. Scavenger receptor class B type I is the major receptor for HDL-cholesterol, and a rare variant abrogates selective HDL-cholesterol uptake, raises HDL-cholesterol, and increases the risk of coronary heart disease [6]. In general, genetic or pharmacological modifications of HDL metabolism and associated compositional changes of the proteome or lipidome of HDL particles may lead to an impaired function of these lipoproteins. Reduced HDL function may also be due to post-translational modifications of proteins or occur as a result of ongoing inflammation [7]. HDL function encompasses several dimensions: cholesterol efflux capacity, vasculoprotective function, anti-inflammatory potential, and antioxidative capacity. Rohatgi et al. [8] demonstrated in a seminal prospective cohort study that HDL-cholesterol efflux capacity predicted incident cardiovascular events independent of traditional risk factors, HDL-cholesterol level, and HDL-particle concentration. According to a modified version of the HDL hypothesis, improving HDL function will lead to a decrease of coronary events [2].

2. Biological HDL-targeted therapies

The HDL hypothesis remains unproven till now. The stringent requirement for proving or refuting this hypothesis is HDL specificity of the intervention, which implies that the causal pathway between the therapeutic intervention and a hard clinical endpoint obligatory passes through HDL. None of the HDL-raising small chemical compounds that have been evaluated till now in randomized phase III trials (niacin, fibrates, torcetrapib, dalcetrapib, evacetrapib) is characterized by HDL specificity. It should be noted that all these drugs were evaluated on a background of the best available therapy including statins. In contrast, infusion therapy of reconstituted HDL particles [9] and human *apo A-I* gene transfer [2] are biological HDL-targeted therapies that are distinguished by HDL specificity. Apo A-I is the major protein component of HDL and plays a critical role in the biological properties of HDL. Reconstituted HDL contain recombinant or purified apo A-I in combination with phospholipids and are a source of lipid-poor pre–β-HDL-like particles that interact efficiently with ATP-binding cassette transporter A1 [2]. The effect of reconstituted HDL on biochemical endpoints, on HDL function, on coronary intravascular ultrasound parameters, and on safety endpoints including hepatotoxicity and renal toxicity has been evaluated in several clinical trials [10,11]. An exhaustive discussion of different coronary intravascular ultrasound trials investigating the effects of biological HDL-targeted therapies is outside the scope of this manuscript. In general, whereas positive effects

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of reconstituted HDL therapy on HDL function have been observed, no single reconstituted HDL infusion therapy study has demonstrated clear regression of atherosclerosis. These observations are entirely consistent with experimental data showing that human *apo A-I* overexpression in mice also fails to induce regression of advanced atherosclerotic lesions [12,13]. Whether regression of atherosclerosis is an acceptable imaging biomarker will be discussed later.

Infusion of lipid-poor pre-β-HDL-like particles in patients with coronary heart disease may induce cholesterol unloading in lesions and/or reduce plaque inflammation and vulnerability. On the other hand, the enzyme lecithin:cholesterol acyltransferase (LCAT) might be rate limiting in patients with coronary heart disease, which often have elevated levels of pre-β-HDL and low levels of large, cholesteryl ester-rich HDL (α_1-HDL) [14]. LCAT catalyzes the production of cholesteryl ester from free cholesterol and phosphatidylcholine. This reaction predominantly occurs on HDL (α-LCAT activity) and to a lesser extent on apo B-containing particles (β-LCAT activity). LCAT plays an essential role in HDL remodeling by promoting the maturation of small discoidal forms of HDL (pre-β-HDL and α_4-HDL) into larger spherical forms of HDL ($\alpha_{1\text{-}3}$-HDL) [14]. A phase 1b, open-label, single-dose escalation study was conducted to evaluate safety, tolerability, pharmacokinetics, and pharmacodynamics of recombinant human LCAT (ACP-501; AstraZeneca) in subjects with stable coronary heart disease and low HDL-cholesterol [14]. HDL-cholesterol 6 h after the two highest doses of 9.0 and 13.5 mg/kg was increased by 36% and 42%, respectively, and remained above baseline for 4 days. ACP-501 potentiated *ex vivo* cholesterol efflux.

3. Surrogate endpoints versus clinically meaningful endpoints

The future of biological HDL-targeted therapies in patients with stable coronary heart disease or with acute coronary syndromes is closely linked to the demonstration of an effect on clinically meaningful primary endpoints (clinical efficacy measures). A surrogate endpoint is an outcome measure used as a substitute for a clinically meaningful endpoint whereby changes induced by a therapy on a surrogate endpoint are expected to reflect changes in a clinically meaningful endpoint. The surrogacy of specific biomarkers in current clinical trials evaluating biological HDL-targeted therapies is a critical issue that should be addressed. With regard to coronary intravascular ultrasound studies evaluating imaging biomarkers, the inherent assumption is that changes in plaque volume are on the causal pathway between intervention and clinical efficacy measure, for example, coronary events. A direct relationship between atheroma progression and regression on coronary intravascular ultrasound and hard clinical events has never been clearly demonstrated [15]. It is perfectly possible that a greater clinical effect is produced by altering plaque composition from lipid-rich to more fibrotic tissue, thus stabilizing the plaque, independent of any change in lumen area or plaque volume. Whereas an unequivocal and clinically important effect of the proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitor evolocumab on top of statins has been recently demonstrated in the FOURIER trial for the primary and key secondary endpoint, the effect of this drug on percent atheroma value in the GLAGOV trial was, although clearly statistically significant, limited [16]. There is a risk of false-negative conclusions about clinical efficacy if the biomarker does not lie in the disease process causal pathway that is meaningfully impacted by the intervention. This might be the case with coronary intravascular ultrasound parameters of plaque volume. Consequently, one of the obvious concerns is that coronary intravascular ultrasound studies evaluating biological HDL-targeted therapies, which fail to reach the primary endpoint, may lead to the erroneous conclusion that no effect on hard clinical endpoints can be expected. The problems surrounding potential surrogate imaging endpoints are also exemplified by data on carotid intima-media thickness. Although carotid intima-media thickness is associated with future cardiovascular disease event risk, regression or slowed progression of carotid intima-media thickness, induced by cardiovascular drug therapies, does not reflect a reduction in cardiovascular events [17]. In general, a strong correlation between a biomarker and a clinical endpoint in natural history observations does not necessarily imply that the biomarker is a (non-validated) surrogate that reasonably likely predicts clinical benefit. Moreover, biomarkers that are strongly correlated with clinical efficacy measures in natural history observations, yet are not in the causal pathway of the disease process, as is the case for carotid intima-media thickness, are likely to provide misleading information about clinical efficacy.

The primary outcome measure in definitive trials should be a clinical event relevant to the patient or an endpoint that measures directly how a patient feels, functions, or survives, where function refers to patients' ability to perform activities in their daily lives [18]. In a less ideal situation, an endpoint can be a validated surrogate for a clinically meaningful endpoint. The central point is that there are no validated surrogate endpoints available for biological HDL-targeted therapies. This is clear from a logical point of view since no trials with a clinically meaningful primary endpoint have been conducted till now and thus, validation has never been performed. In contrast, in the setting of hypolipidemic therapies, low-density lipoprotein (LDL) cholesterol is a reasonable surrogate endpoint because based on prior art, a strong linear relationship has been demonstrated between absolute LDL-cholesterol reduction (mmol/L) and reduction of the rate of major cardiovascular events (%). However, even a validated surrogate endpoint constitutes an imperfect substitute of a clinically meaningful endpoint since such endpoint unlikely captures the complete effect of the intervention. Specifically, the intervention may modify causal pathways that have an impact on the endpoint but are unrelated to the biomarker that serves as a surrogate endpoint. If we assume that parameters of HDL functionality are on the causal pathway of atherosclerotic vascular disease, false-positive conclusions about clinical efficacy may arise if HDL function captures substantial effects of an intervention on one causal pathway of the disease process, while the intervention has an inadequate impact on other principal causal pathways.

4. Conclusion

The current state of the art on the effects of HDL-targeted therapies is faced with a lack of a validated surrogate endpoint and suffers from uncertainty with regard to non-validated surrogates that reasonably likely predict clinical outcomes. This may lead to false-negative and false-positive conclusions.

A statistical power calculation for a trial with a clinically meaningful primary endpoint is critically dependent on the assumption with regard to the expected hazard ratio. The required number of events is inversely proportional to (log (hazard ratio))^2. The required number of events is, for example, 2.38-fold higher for a hazard ratio of 0.9 compared to a hazard ratio of 0.85 for the same level of statistical significance and statistical power. In the absence of any prior art, it is very hard to predict the hazard ratio.

Taken together, in light of all these considerations, it is far from evident to prove or to refute the HDL hypothesis in the setting of atherosclerotic vascular disease.

5. Expert opinion

One approach is to restrict biological HDL-targeted therapies to patients with familial primary hypoalphalipoproteinemia, which concerns a relatively low number of patients. In these subjects, a biochemical endpoint is acceptable since the objective of the intervention is to treat a protein deficiency. Lack of detectable plasma apo A-I can be due to DNA deletions, rearrangements, or nonsense or frameshift mutations within the *APOA1* gene and results in premature coronary heart disease [19]. This disorder is a clear target for adeno-associated viral serotype 8 (AAV8)-mediated human *apo A-I* gene therapy. Familial LCAT deficiency is characterized by extremely low HDL-cholesterol, corneal opacities, anemia, and progressive renal disease but no marked increase of coronary heart disease [19]. This disorder may be treated with recombinant LCAT protein therapy.

Another option is to consider new therapeutic areas for HDL-targeted interventions [2]. Pleiotropic effects of HDL might be translated in clinically significant effects in strategically selected therapeutic areas that are not directly related to native coronary artery disease. In particular, HDL-targeted therapies might be useful in the setting of critical illness [2] via its anti-inflammatory and antioxidative effects and in the setting of heart failure (HF). HF is a growing public health problem, the leading cause of hospitalization, and a major cause of mortality. Approximately 50% of chronic HF patients have HF with reduced ejection fraction (HFrEF) and 50% suffer from HF with preserved ejection fraction (HFpEF). As the population ages, HFpEF will continue to be a growing public health problem. In contrast to advances in the treatment of patients with HFrEF, drug strategies with strong evidence in HFrEF have proved unsuccessful in HFpEF and the mortality in patients with HFpEF has remained unchanged. The rationale for HDL-targeted therapies in this setting is based on biological, epidemiological, and experimental evidence [20]. Specifically, HDL-targeted therapies improve diastolic function and exert antifibrotic effects [20].

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Declaration of interest

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